10/11/17

Wednesday, October 11, 2017 2:42 PM

Who's in Lab:

Just met up with Tyler because the sequencing results are somewhat incomplete.
The LC1853 (DNA Ligase) and LC1539 (DNA polymerase) were sent to the UIUC Core sequencing Facility on 10/4/17.

While the results do indicate that the something similar to our inserts are both in our JOE vector plasmid, we cannot be sure until we get the entire sequence verified. We have issues with the endings of the sequence. While the endings of a sequence are never guaranteed a good seqeuncing read because the process of sequencign the DNA, our fault was that we used primers designed specifically to the genes themselves as opposed to primers that were designed for the vector JOE.

We need to send in results with REV and FOR primers for JOE (we already have these in the –20F freezer) to have sequecning begin updtream of our gene of interest.

FOR LC 1853,

We are missing DNA seqwuencing data for the middle of the gene. Hence we need to design FOR and REV primers for the middle of the gene to get sequence for that.

LC1539 (DNA Ligase):

The middle part of the sequence is known and the linker SSB protein DNA (Sulfolobus solfataricus) had 100% identicalness when we blasted the translated PROTEIN sequence of the nucleotide returned from UIUC core with the protein sequence we had found.

The DNA polymerase sequence itself had 96% identicalness with the sequence we had found

```
                     /db_xref="CDD:99919"
ORIGIN
        1 mildvdyite egkpvirlfk kengkfkieh drtfrpyiya llrddskiee vkkitgerhg
       61 kivrivdvek vekkflgkpi tvwklylehp qdvptirekv rehpavvdif eydipfakry
      121 lidkglipme geeelkilaf dietlyhege efgkgpiimi syadeneakv itwknidlpy
      181 vevvsserem ikrflriire kdpdiivtyn gdsfdfpyla kraeklgikl tigrdgsepk
      241 mqrigdmtav evkgrihfdl yhvitrtinl ptytleavye aifgkpkekv yadeiakawe
      301 sgenlervak ysmedakaty elgkeflpme iqlsrlvgqp lwdvsrsstg nlvewfllrk
      361 ayernevapn kpseeeyqrr lresytggfv kepekglwen ivyldfraly psiiithnvs
      421 pdtlnlegck nydiapqvgh kfckdipgfi psllghllee rqkiktkmke tqdpiekill
      481 dyrqkaikll ansfygyygy akarwyckec aesvtawgrk yielvwkele ekfgfkvlyi
      541 dtdglyatip ggeseeikkk alefvkyins klpglleley egfykrgffv tkkryavide
      601 egkvitrgle ivrrdwseia ketqarvlet ilkhgdveea vrivkeviqk lanyeippek
      661 laiyeqitrp lheykaigph vavakklaak gvkikpgmvi gyivlrgdgp isnrailaee
      721 ydpkkhkyda eyyienqvlp avlrilegfg yrkedlryqk trqvgltswl nikks
//
```

https://www.ncbi.nlm.nih.gov/protein/WP_011011325.1

LC1853:

Reverse LC1853 primer sequence read nucleotide blast results:

Score	Expect	Identities	Gaps	Strand
1306 bits(707)	0.0	787/822(96%)	27/822(3%)	Plus/Minus

```
Query  1431  TTT-CCA-GATAG-CAG-AAAAA-TT-GGGAC-TC-GAC-AAA-CGTATAAT-CG-AAAA  1478
             ||| ||| ||||| ||| ||||| || ||||| || | | ||| ||| |||| || ||||
Sbjct  916   TTTCCCAGGATAGCCAGAAAAAATTTGGGGACNTCGGNCNAAACCGTNTAATCCGAAAAA  857

Query  1479  CG-CCA-GGTCGAGACTCTCTC-GGGAGGAGATGGAA-CTCGAGGGACT-CATAAGGTCT  1533
             || ||| ||||||||||||||| ||||||||||||| |||||||||||| ||||||||||
Sbjct  856   CGCCCAGGGTCGAGACTCTCTCCGGGAGGAGATGGAANCTCGAGGGACTCCATAAGGTCT  797

Query  1534  CTCCACGAGAAGATCTCGCTTCTCgaagaggagaagagaaaactcc-agaaagaacgcga  1592
             ||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  796   CTCCACGAGAAGATCTCGCTTCTCGAAGAGGAGAAGAGAAAACTCCCAGAAAGAACGCGA  737

Query  1593  agagtacatgaaactgagagagaaatacgaagaagaCTACAAAAAGCTGAGAAGAATGAA  1652
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  736   AGAGTACATGAAACTGAGAGAGAAATACGAAGAAGACTACAAAAAGCTGAGAAGAATGAA  677

Query  1653  AATAGAAGAGTTCGACAAAGAGCTGAGGGAGCTCAACGATTACATCAGAAAGGTCAAGAA  1712
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  676   AATAGAAGAGTTCGACAAAGAGCTGAGGGAGCTCAACGATTACATCAGAAAGGTCAAGAA  617

Query  1713  GGAACTCGATCAGGCGATACACGTGGCAAAAACTGGCAGCGTTGACgagatgagagaagc  1772
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  616   GGAACTCGATCAGGCGATACACGTGGCAAAAACTGGCAGCGTTGACGAGATGAGAGAAGC  557

Query  1773  ggtgaagacgatagagaaagagaagaaagatctggagcaaaagagaatcgaagaagcgac  1832
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  556   GGTGAAGACGATAGAGAAAGAGAAGAAAGATCTGGAGCAAAAGAGAATCGAAGAAGCGAC  497

Query  1833  cgaagaagaaataaaaCCCGGAGATCACGTGAAAATGGAAGGTGGAACCTCTGTGGGGAA  1892
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  496   CGAAGAAGAAATAAAACCCGGAGATCACGTGAAAATGGAAGGTGGAACCTCTGTGGGGAA  437

Query  1893  GGTCGTTGAGGTGAAAAGTGGCACCGCCCTTGTTGACTTTGGCTTTCTCAGATTGAAGGT  1952
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  436   GGTCGTTGAGGTGAAAAGTGGCACCGCCCTTGTTGACTTTGGCTTTCTCAGATTGAAGGT  377

Query  1953  GCCCGTGTCGAAACTGagaaagacgaaaaaagaagagaagaaagaaaCTTCAACGTTCTC  2012
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  376   GCCCGTGTCGAAACTGAGAAAGACGAAAAAAGAAGAGAAGAAAGAAACTTCAACGTTCTC  317

Query  2013  TTACAAACCTTCGAGCTTCAGAACGGAAATAGACATAAGGGGTATGACGGTTGAAGAAGC  2072
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  316   TTACAAACCTTCGAGCTTCAGAACGGAAATAGACATAAGGGGTATGACGGTTGAAGAAGC  257

Query  2073  GGAGCCGGTTGTGAAGAAGTTCATCGATGACCTGATGATGAA-CGGCATCAGCAA-GGGA  2130
             ||||||||||||||||||||||||||||||||||||||||||| ||||||||||| ||||
Sbjct  256   GGAGCCGGTTGTGAAGAAGTTCATCGATGACCTGATGATGAAACGGCATCAGCAANGGGA  197

Query  2131  TACATAATACACGGAAA-GGGCA-CCGGAAAGCTCGCATCTGGAGTA-TGGGA-AATAC-  2185
             |||||||||||||||||| ||||| |||||||||||||||||||||| |     ||||
Sbjct  196   TACATAATACACGGAAATGGGCANCCGGAAAGCTCGCATCTGGAGTACTCCNTTAATANG  137

Query  2186  TGAGAAAGGACAAAA-GAGTGGTTTCTTTCA-GATTCGGAAC  2225
             |||||||||||||||| |||||||||||||  ||||||||||
Sbjct  136   TGAGAAAGGACAAAACGAGTGGTTTCTTTCCNGATTCGGAAC  95
```

Score	Expect	Identities	Gaps	Strand
1262 bits(683)	0.0	757/791(96%)	24/791(3%)	Plus/Plus

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Query  49    AAGAAATACGCGC-TCTCTGACCT-GGGAAGAAAACATCT---GGACACTCTCAAACCGA  103
             |||||||||||||| |||||||||| |||||||      | |   ||||||||||||||||
Sbjct  114   AAGAAATACGCGCGTCTCTGACCTNGGGAAG-NCNTNTTTNNNGGACACTCTCAAACCGA  172

Query  104   CGGTGAATCCGTGGGACGAGCTCGAACTCGTGGAGGAGCTTCTGAACTATTTTAACAGGT  163
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  173   CGGTGAATCCGTGGGACGAGCTCGAACTCGTGGAGGAGCTTCTGAACTATTTTAACAGGT  232

Query  164   GGGGAGAGCCTCCCATAAAGGGATTGAACGATATCTCTCAGGAAGTGGAGAAGGTGAAGT  223
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  233   GGGGAGAGCCTCCCATAAAGGGATTGAACGATATCTCTCAGGAAGTGGAGAAGGTGAAGT  292

Query  224   CCGGCTCCCCTCTGGAACCGTGGGAACTTCTTCGTGTCTCCGTGTTTCTCGAAGGCTGTG  283
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  293   CCGGCTCCCCTCTGGAACCGTGGGAACTTCTTCGTGTCTCCGTGTTTCTCGAAGGCTGTG  352

Query  284   ACATCCTGAAGAAAGAATTTGAAAAGCGTGAATACAGCAGACTCAAAGAGACCTTCTCCA  343
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  353   ACATCCTGAAGAAAGAATTTGAAAAGCGTGAATACAGCAGACTCAAAGAGACCTTCTCCA  412

Query  344   GGCTCAGCTCCTTCAGAGAGTTCGTAGAAGAGGTGAACAGGTGCATAGAACAGGATGGAG  403
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  413   GGCTCAGCTCCTTCAGAGAGTTCGTAGAAGAGGTGAACAGGTGCATAGAACAGGATGGAG  472

Query  404   AGATCTCGGACCGTGCGAGTCCCAGATTGAGGGAGATCAGAACGGAAAAGAAGCGTCTTT  463
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  473   AGATCTCGGACCGTGCGAGTCCCAGATTGAGGGAGATCAGAACGGAAAAGAAGCGTCTTT  532

Query  464   CCAGCGAGATAAAGAGAAAGGCCGATGATTTCGTCAGGACGCACTCTCAGATCCTTCAGG  523
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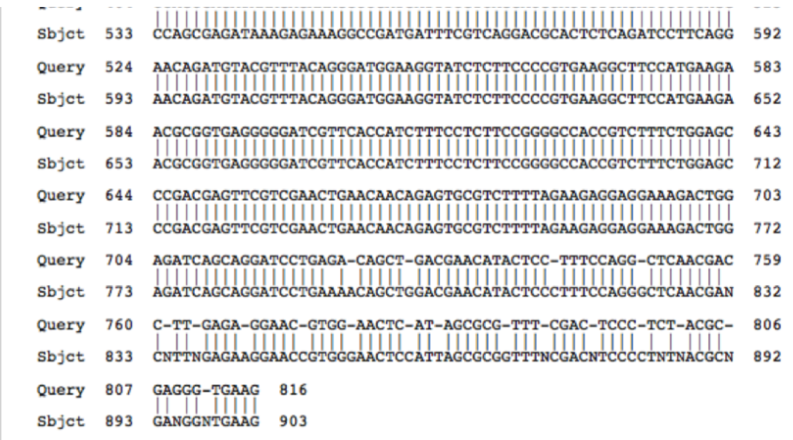

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                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  533  CCAGCGAGATAAAGAGAAAGGCCGATGATTTCGTCAGGACGCACTCTCAGATCCTTCAGG  592

Query  524  AACAGATGTACGTTTACAGGGATGGAAGGTATCTCTTCCCCGTGAAGGCTTCCATGAAGA  583
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  593  AACAGATGTACGTTTACAGGGATGGAAGGTATCTCTTCCCCGTGAAGGCTTCCATGAAGA  652

Query  584  ACGCGGTGAGGGGGATCGTTCACCATCTTTCCTCTTCCGGGGCCACCGTCTTTCTGGAGC  643
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  653  ACGCGGTGAGGGGGATCGTTCACCATCTTTCCTCTTCCGGGGCCACCGTCTTTCTGGAGC  712

Query  644  CCGACGAGTTCGTCGAACTGAACAACAGAGTGCGTCTTTTAGAAGAGGAGGAAAGACTGG  703
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  713  CCGACGAGTTCGTCGAACTGAACAACAGAGTGCGTCTTTTAGAAGAGGAGGAAAGACTGG  772

Query  704  AGATCAGCAGGATCCTGAGA-CAGCT-GACGAACATACTCC-TTTCCAGG-CTCAACGAC  759
            |||||||||||||||||| | ||||| |||||||||||||| |||||||| |||||||| 
Sbjct  773  AGATCAGCAGGATCCTGAAAACAGCTGGACGAACATACTCCCTTTCCAGGGCTCAACGAN  832

Query  760  C-TT-GAGA-GGAAC-GTGG-AACTC-AT-AGCGCG-TTT-CGAC-TCCC-TCT-ACGC-  806
            | || |||| ||||| |||| ||||| || |||||| ||| |||| |||| | | |||| 
Sbjct  833  CNTTNGAGAAGGAACCGTGGGAACTCCATTAGCGCGGTTTNCGACNTCCCCTNTNACGCN  892

Query  807  GAGGG-TGAAG  816
            || || |||||
Sbjct  893  GANGGNTGAAG  903
```

What's next:

--Get lysozyme
--Do we have dNTPs?
--Dp we have DTT chemical?
--Do we have the PEG polymer buffer?
--We used a Taq Q5 polymerase, so our PCR should have been good, This gives us some confidence in our contructs.

--We will use freeze and thaw method to lyse cells
    --liquid nitrogen??
    --Otherwise it's dry ice with isopropanol
--We will do colony PCRs to verify our results and we will also do sequencing...

--We need to design PRIMERS!!!!

--Use as checklist for what materials we need:
https://www.researchgate.net/post/Does_someone_have_a_protocol_for_home_made_Gibson_assembly_master_mix
http://miller-lab.net/MillerLab/protocols/molecular-biology-and-cloning/gibson-assembly/

- -send Short logo
- -Who's coming to Genome Day, by MOnday
- 1-5pm on Nov 4th,2017
  Send Sigma Aldrich order